Applicants' Remarks/Arguments

I. Status

Claims 1, 3-19 and 21-36 have been examined. Applicants have cancelled claims 1, 11, 19, 20 and 29 in favor of new claims 37-39 and have additionally introduced new claims 40-54. Accordingly, claims 3-10, 12-18, 21-28, and 30-54 are presently pending and under Examination.

New claim 37 is directed to an aqueous gel consisting essentially of a tris-borate buffer solution and a dissolved hydrophilic polymer that imparts structural framework and rigidity to the gel. Support for the recitations relating to the composition of the tris-borate buffer can be found page 8, line 24 – page 9, line 4. Support for the recitation relating to "structural framework and rigidity" can be found at page 12, lines 23-26. Support for the recitation that the hydrophilic polymer is dissolved in the tris-borate buffer solution can be found at page 18, lines 26-28. Support for the recitation that the gel results from the entanglement of polymer molecules can be found page 12, line 26 – page 13, line 12. Support for the recitation of alcohol can be found in original claim11.

New claim 38 is directed to a capillary electrophoresis system comprising a capillary tube containing an aqueous gel. Support for the recitation relating to "structural framework and rigidity" can be found at page 12, lines 23-26. Support for the recitations relating to the composition of the tris-borate buffer can be found page 8, line 24 – page 9, line 4. Support for the recitation that the hydrophilic polymer is dissolved in the tris-borate buffer solution can be found at page 18, lines 26-28. Support for the recitation that the gel results from the entanglement of polymer molecules can be found page 12, line 26 – page 13, line 12. Support for the recitation of alcohol can be found in original claim11.

New claim 39 is directed to a capillary electrophoresis system comprising a capillary tube containing an aqueous gel. Support for the recitation relating to "structural framework and rigidity" can be found at page 12, lines 23-26. Support for the recitations

relating to the composition of the tris-borate buffer can be found page 8, line 24 – page 9, line 4. Support for the recitation that the hydrophilic polymer is dissolved in the tris-borate buffer solution can be found at page 18, lines 26-28. Support for the recitation that the gel results from the entanglement of polymer molecules can be found page 12, line 26 – page 13, line 12. Support for the recitation that the gel forms a dynamic coating on the inner surface of said capillary tube can be found at page 18, lines 26-28. Support for the recitation of alcohol can be found in original claim11.

New claims 40-54 parallel claims 21-28 and 30-36, respectively, and are supported by the original specification and by such claims.

Applicants have amended claims 7 and 25 to provide improved antecedent basis, as requested by the Examiner. Applicants have additionally clarified that the aqueous gel medium of the present invention comprises entangled molecules of a hydrophilic polymer (please see page 12, line 26 – page 13, line 12, and particularly, page 12, line 26-28) in the presence of the other components of the medium. Applicants have additionally clarified the purpose of the reducing agents. Support for these recitations may be found at page 2, lines 1-4 of the specification,

No new matter has been added by any of the requested amendments.

Applicants greatly appreciate the courtesy of the Examiner and her supervisor in granting a personal interview to the undersigned on October 4, 2007. At the interview, Applicants discussed the scope and content of U.S. Patent No. 5,370,777, and recitations that could be introduced into the claims to more clearly define the patentable subject matter of the present invention.

II. The Invention

The present invention relates to a novel aqueous electrophoresis gel medium and a capillary electrophoresis system comprising such a medium.

The ability to separate analytes has, in the past, been impacted by several problems. For example, only certain polymers are capable of separating polynucleotides and proteins (please see page 6, lines 21-23 of the Specification). Significantly, as analyte size increases, relative differences in charge diminish (please see page 2, lines 11-20; U.S. Patent No. 5,370,777 (**Guttman *et al.* '777**) at column 2, lines 16-22). Accordingly, the art has recognized the desirability of employing detergents (such as sodium dodecyl sulfate) to denature large analytes (such as proteins and polypeptides) so that disparities in their effective charges will not distort the rate with which such molecules migrate through the electrophoretic matrix (please see **Guttman *et al.*** '777 at column 2, lines 42 - 47). Such treatment, however, entails the use of a ***charged*** medium possessing a pH greater than 3. The use of such a medium causes the silanol groups of glass capillary tubes to ionize (please see page 6, lines 29-30 of the Specification). Aqueous gel media containing negatively charged detergents bind poorly to the capillary surface under such conditions (please see page 6, line 21 – page 7, line 2). Such poor binding causes undesirable electroendoosmotic flow and analyte-wall interactions that distort the electrophoretic separation (please see, page 4, lines 14-15 of the Specification).

The art teaches that these problems may be addressed using capillary tubes having a ***permanently affixed*** coating on their internal surface (see, e.g., **Guttman *et al.*** '777 at column 6, lines 44-51, column 17, lines 8-18; please see Declaration of Dr. Liu at Paragraph 4C). But, this solution suffers from the problem that sample throughput is decreased and the coatings are often unstable under acidic or basic conditions. Moreover, crosslinking such coatings to the surface of the capillary tube can be time-consuming and expensive.

The present invention provides an alternative aqueous gel composition that may be used in initially uncoated capillary tubes because it forms a ***dynamic*** coating on the internal surface of capillary tubes and thereby renders them suitable for capillary

electrophoresis (please see the Specification at page 18, lines 26-28; Declaration of Dr. Liu at Paragraph 4D).

III. The Rejections Pursuant to 35 U.S.C. § 112, Second Paragraph

Claims 7 and 25 have been rejected pursuant to 35 U.S.C. § 112, second paragraph, as failing to point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner refers to a lack of antecedent basis for the limitation "said reducing agent" in lines 1-2 of Claims 7 and 25. The claims have been amended to state "said metal ion chelator", which correctly identifies the compound in claims 7 and 25. Support for these amendments is found in claims 6 and 24. In light of Applicants' amendments and the above remarks, Applicants respectfully submit that the rejection of claims 7 and 25 pursuant to 35 U.S.C. § 112, second paragraph may now be properly withdrawn.

IV. The Rejections Pursuant to 35 U.S.C. § 103(a)

A. The Rejection of Claims 1, 3-9, 11-13, 16-19, 21-27, 29-31 and 34-36.

Claims 1, 3-9, 11-13, 16-19, 21-27, 29-31 and 34-36 are rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,370,777 (**Guttman *et al.* '777**). **Guttman *et al.* '777** is stated to disclose an aqueous gel medium, comprising a non-cross-linked hydrophilic polymer, for facilitating the electrophoretic separation of analytes present in a sample. The Examiner has advised that **Guttman *et al.* '777** discloses an invention that is indistinguishable from that previously claimed by Applicants. Applicants respectfully traverse the rejection and request reconsideration.

1. The Aqueous Gel of the Present Invention Is Not Obvious in Light of Guttman *et al.* '777

Applicants have amended the claims of the application to more clearly describe the nature of the gel medium of the present invention, and respectfully submit that the

presently claimed invention is distinct from, and not obvious in light of **Guttman *et al.*** '777.

Applicants gel medium consists essentially of a tris-borate buffer solution and a dissolved hydrophilic polymer. As the Examiner will recognize, **Guttman *et al.*** '777 teaches the use of a tris-borate buffer solution ***only*** in concert with a chaotropic denaturation agent (i.e., 6-8 M urea or 3-8 M urea in the presence of 20-40% of a non-urea denaturing agent or 98% of a non-urea denaturing agent capable of disrupting hydrogen bonding; column 10, lines 21-54). Applicants amended claims do not include such a chaotropic denaturation agent.

Applicants respectfully submit that in light of the clear teaching of **Guttman *et al.*** '777 to *include* a chaotropic denaturation agent in compositions having tris-borate buffers, the *elimination* of this component would not have been obvious.

The Examiner has advised that the pH ranges recited in claim 1 would have been *prima facie* obvious in light of **Guttman *et al.*** '777 (which is alleged to teach a pH range of 8.0-10.0, more expressly 8.0 – 8.5, and more specifically a pH of 8.3). Applicants respectfully submit that those of ordinary skill would not have drawn this conclusion. It is submitted that **Guttman *et al.*** '777does not teach, suggest, motivate or predict the pH of a gel electrophoresis medium that is ***not*** permanently affixed to the capillary wall.

The Examiner has advised that **Guttman *et al.*** '777 discloses the inclusion of reducing reagents such as dithiothreitol and 2-mercaptoethanol with the sample, and has suggested that such reducing reagents, by virtue of their small size, would diffuse into the gel material and thus serve to keep analytes in a reduced form. Applicants respectfully request reconsideration of this conclusion.

It is submitted that those of ordinary skill would have concluded that **Guttman *et al.*** '777 teaches the use of a reducing reagent ***solely*** for the purpose of sample preparation; i.e., "***before*** introduction into the capillary column" (see **Guttman *et al.***

'777, column 18, lines 41-42, emphasis added). It is therefore respectfully submitted that those of ordinary skill would not have drawn such a conclusion for at least two reasons:

1. The conclusion fails to address the fact that analyte molecules, by virtue of their size and charge will migrate in capillary electrophoresis and thus will separate ***away*** from the uncharged reducing reagents (please see page 2, lines 6-7 of the Specification). Accordingly, such reducing reagents, even if provided by **Guttman *et al.* '777**, would be ***incapable*** of functioning to ***keep*** analytes in a reduced form, as is presently claimed, since the analytes and the reducing reagents *would be continuously migrating further and further apart*. As the Examiner will appreciate, even if charged reducing reagents were employed, such molecules would migrate faster than the larger protein analytes and would thus also separate from such analytes.
2. The formation of disulfide bonds is chemically favored unless the environment of the analyte is a reducing one, such as that created when a reducing agent is present. The Examiner has proposed that **Guttman '777's** inclusion of reducing reagents such as dithiothreitol and 2-mercaptoethanol would create and maintain such a reducing environment. However, since any interaction between these reducing agents and the disulfide bonds upon which they would act would be a ***non***-covalent interaction, thermodynamic considerations would cause the reduced disulfide bonds to simply re-form as the analyte and reducing agent migrate away from each other. The –SH groups formed by interaction with a reducing agent would thus ***not*** serve to ***keep*** the analyte in a reduced form.

In light of the active and/or differential mobility of the analytes, it is respectfully submitted that the method of **Guttman *et al.* '777** would provide no means for ***maintaining*** the concentration of such reducing reagents at a level sufficient to ***"keep"*** analytes in a reduced form, as is presently claimed. Applicants therefore submit that those of ordinary skill would not have found it obvious in light of **Guttman *et al.* '777** to

have included reducing reagent(s) that function to help ***keep*** protein analytes in a reduced form ***within*** the employed aqueous gel medium. Thus, the improvement provided by the present invention is more than the predictable use of prior art elements according to their established functions. Accordingly, Applicants submit that **Guttman *et al.* '777** does not render the presently claimed gel medium obvious.

2. The Electrophoretic Systems of the Present Invention Are Not Obvious in light of Guttman *et al.* '777

Claim 38, and its dependent claims, are submitted to be patentable over **Guttman *et al.* '777** at least in part because **Guttman *et al.* '777** provides no teaching, suggestion, motivation or prediction of the aqueous gel composition recited in the claim.

Regarding claim 39 and its dependent claims, as the Examiner will note, **Guttman *et al.* '777** teaches "capillary columns comprising combinations of the following: (1) a bifunctional agent which is adsorbed to the inner wall of the capillary column; (2) a gel composition copolymerized with the bifunctional agent; (3) a hydrophilic polymer adsorbed onto the polyacrylamide gel; and (4) a separation composition substantially interspersed throughout the remainder of the column" (**Guttman *et al.* '777** at column 6, lines 44 – 51).

Thus, **all** of **Guttman *et al.* '777's** proposed electrophoretic systems comprise ***at least*** a coating of a bifunctional crosslinking agent **covalently and permanently affixed** to the internal surface of the capillary tube (please see, **Guttman *et al.* '777** at column 6, lines 44 – 51). The present invention is predicated, in part, upon the recognition that by dissolving a hydrophilic polymer into a high concentration tris-borate buffer, a separation medium is produced which suppresses electroosmotic flow and reduces analyte-surface interactions through the formation of a ***dynamic*** coating on the internal surface of the capillary tube, and which therefore can be used in initially uncoated capillary tubes in capillary electrophoresis (please see the Specification at page 18, lines 26-28; Declaration of Dr. Liu at Paragraph 4D). Applicants respectfully submit that **Guttman *et al.* '777** fails to teach, suggest, motivate or predict such an electrophoretic system.

Applicants respectfully draw the Examiner's attention to the fact that **Guttman *et al.* '777** discloses dynamic crosslinking as an alternative to covalent crosslinking as a means for forming a gel. Such disclosure is distinct from and not relevant to the **Guttman *et al.* '777's** disclosed ***coating***. In this regard, the Examiner's attention is respectfully directed to **Guttman *et al.* '777** clear assertion of the ***permanence*** of the disclosed coating ***and its desirability***:

> "Because the bifunctional agent is presumptively adsorbed to the column via ionic forces; the polymer gel is crosslinked to the bifunctional agent; and the hydrophilic polymer is adsorbed to the polymer gel, these components are intended to be "permanently" affixed to the inner wall of the capillary column. The separation compositions, on the other hand, are not intended to be "permanently" affixed to the hydrophilic polymer. Accordingly, these compositions can be removed from the column such that the column can be regenerated. Thus, rather than having to replace the entire column after a series of analytical runs, an investigator can remove the separation composition from the column and regenerate that same column using another separation composition."
>
> **Guttman *et al.* '777**, column 17, lines 8-22; see, also, Example III.

As discussed on page 12, line 26 – page 13, line 7 of the present Specification, Applicants' recognition of a gel capable of forming a dynamic coating was an ***unexpected*** result. In this regard, the Examiner's attention is respectfully directed to **Figure 2** (lower two curves) of the Application which shows the poor resolution of capillary electrophoresis when conducted using hydrophilic polymers and uncoated capillary tubes. In contrast, the upper curve of **Figure 2** shows the capability of the aqueous medium of the present invention to act as a molecular sieve in capillary electrophoresis with uncoated capillary tubes.

The Examiner has advised that the Office interprets **Guttman *et al.* '777** as teaching the use of both permanently coated capillary tubes and uncoated capillary tubes. Applicants respectfully submit that **Guttman *et al.* '777** does not teach, suggest, motivate or predict, a capillary electrophoresis system comprising a ***dynamically*** coated capillary

tube. Thus, the improvement provided by the present invention is more than the predictable use of prior art elements according to their established functions. Accordingly, Applicants submit that **Guttman *et al.* '777** does not render the presently claimed capillary electrophoresis system obvious. In light of Applicants' amendments and the above remarks, Applicants respectfully submit that the rejection of claims 1, 3-9, 11-13, 16-19, 21-27, 29-31 and 34-36 under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,370,777 (**Guttman *et al.* '777**) may now be properly withdrawn.

B. The Rejection of Claims 10 and 28

Claims 10 and 28 have been rejected pursuant to 35 U.S.C. § 103(a) as obvious to one of ordinary skill in light of U.S. Patent No. 5,370,777 (**Guttman *et al.* '777**) in view of "Dextran Product Information", Sigma-Adrich (2001) found at http://www.sigmaaldrich.com/sigmaaldrich/product_information_sheet/d5376pis.pdf. The Dextran Product Information sheet is cited as evidence that commercially available dextran possesses a non-cross-linked structure composed of approximately 95% alpha-D-(1-6) linkages. Applicants respectfully traverse and request reconsideration.

Applicants note that although the Dextran Product Information sheet discloses the nature and percentage of the dextran linkages, and indicates that Dextran of MW 2,000 kDa is commercially available, it does not appear to teach, suggest, motivate or predict the use of dextran compositions having the molecular weight recited in applicants' claims, nor does it remedy the deficiencies of **Guttman *et al.* '777** with respect to the non-obviousness of the presently claimed invention. In this regard, not a single reference given on the Product Information Sheet teaches the use of Dextran either at the MW of the present application, or for use in capillary gel electrophoresis. **Guttman *et al.* '777** does not remedy this deficiency, since it provides no basis for concluding either the inherency of dextran molecular weights or that the dextran employed by **Guttman *et al.* '777** meets the nature and percentage of the dextran linkages recited in the claims.

It is submitted that the combined teachings, suggestions, motivations, or predictions of the references thus fail to render the claimed invention obvious. Applicants respectfully submit that claims 10 and 28 comprise the recitations of previously amended claims 1 and 19, and accordingly are patentable for the reasons stated with respect to such claims the rejections based on **Guttman *et al.* '777**. Thus, the improvement provided by the present invention is more than the predictable use of prior art elements according to their established functions. Accordingly, Applicants respectfully submit that the rejection of claims 10 and 28 pursuant to 35 U.S.C. § 103(a) in light of U.S. Patent No. 5,370,777 (**Guttman *et al.* '777**) as combined with "Dextran Product Information," Sigma-Adrich (2001) may now be properly withdrawn.

C. The Rejection of Claims 14-15 and 32-33

Claims 14-15 and 32-33 have been rejected pursuant to 35 U.S.C. § 103(a) as obvious to one of ordinary skill in light of U.S. Patent No. 5,370,777 (**Guttman *et al.* '777**) in view of U.S. Patent No. 5,213,669 (**Guttman '669**). **Guttman '669** is stated to teach an aqueous gel medium having an alcohol that is glycerol. Applicants respectfully traverse and request reconsideration.

Applicants submit that claims 14-15 and 32-33 comprise the recitations of claims 37-39, and accordingly are patentable for the reasons stated with respect to such claims the rejections based on **Guttman *et al.* '777**. **Guttman '669** does not teach the use of tris-borate buffers. Accordingly, the hypothetical combination of **Guttman *et al.* '777** **Guttman '669** would require selectively choosing to employ the alcohols taught by **Guttman '669** while eschewing the buffer systems taught by **Guttman '669** as necessary when using such alcohols; it would further require employing the tris-borate buffer taught by **Guttman *et al.* '777** while eschewing the chaotropic denaturation agent taught by **Guttman *et al.* '777** as necessary when using such buffer. Applicants respectfully submit that such substitutions are not obvious and that the combination of **Guttman *et al.* '777** and **Guttman '669** fails to teach, suggest, motivate or predict the use of a tris borate buffer in the aqueous gel or capillary electrophoresis systems presently claimed.

Additionally, Applicants note that the compositions recited within the presently claimed inventions provide unexpectedly better results than those obtainable using the compositions of **Guttman '669** (please see Example 5 of the present application). Thus, the improvement provided by the present invention is more than the predictable use of prior art elements according to their established functions. Accordingly, in light of Applicants' amendments and the above remarks, Applicants respectfully submit that the rejection of claims 4-15 and 32-33 pursuant to 35 U.S.C. § 103(a) in light of U.S. Patent No. 5,370,777 (**Guttman *et al.* '777**) as combined with U.S. Patent No. 5,213,669 (**Guttman '669**) may now be properly withdrawn.

V. Concluding Remarks

Applicants submit that the present response is complete and complies with the requirements of 35 U.S.C. §121. The Application is believed to be in condition for Allowance and early notice of such favorable action is respectfully requested. Should the Examiner have any questions regarding the subject invention or its patentability, Applicants encourage the Examiner to contact the undersigned to answer such questions or provide any desired additional information.

Respectfully Submitted,

Date: **October 11, 2007**

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